Issued May 1, 1913.

U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF PLANT INDUSTRY—BULLETIN NO. 281.
WILLIAM A. TAYLOR, *Chief of Bureau.*

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BY

L. L. HARTER, *Pathologist,*

AND

ETHEL C. FIELD, *Scientific Assistant,*
Cotton and Truck Disease and Sugar-Plant Investigations.

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BUREAU OF PLANT INDUSTRY.

Chief of Bureau, WILLIAM A. TAYLOR.
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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., December 20, 1912.

Sir: I have the honor to transmit herewith and to recommend for publication as a bulletin of this Bureau a manuscript entitled "A Dry Rot of Sweet Potatoes Caused by Diaporthe Batatatis," by Mr. L. L. Harter, Pathologist, and Miss Ethel C. Field, Scientific Assistant, Office of Cotton and Truck Disease and Sugar-Plant Investigations.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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A DRY ROT OF SWEET POTATOES CAUSED BY DIAPORTHE BATATATIS.

INTRODUCTION.

In 1890 Halsted [1] briefly called attention to a disease of the sweet potato (*Ipomoea batatas*) in New Jersey which he attributed to an undescribed species of Phoma. So far as we have been able to determine, this citation constitutes the first published reference to the disease. To this organism Ellis and Halsted assigned the name *Phoma batatae*.[2] Halsted stated in his discussion of the disease that the rot is only occasionally found, and for that reason he did not consider it of much importance. As a precautionary measure, however, he recommended that all decayed potatoes as well as the rubbish after each crop should be removed from the field and destroyed.

Halsted did not regard the disease as serious at that time, which probably explains why he did little or no more work with it. During the 22 years which have elapsed since this disease was first discovered and described, no additional work has been done with it.

It is believed by the writers, after a thorough study of this disease, that the economic importance of the dry-rot has been obscured by two very serious and prevalent diseases—the stem-rot and the black-rot. It has been further obscured by the fact that there are no external evidences of the organism in the field, it being detected only on the dead vines in the field or on the roots in the storage house a month or more after the crop is harvested. In view of these facts, injury which has been occasioned by the dry-rot organism is frequently attributed to other diseases, such as stem-rot or even to drying up due to natural causes.

Specimens of dry-rot have been received from a number of States. In 1910 and during the spring and summer of 1911 diseased plants were collected in New Jersey and Virginia. The disease was reported by Dr. R. P. Hibbard, then pathologist of the Mississippi Agricultural Experiment Station, to be present in Mississippi in 1908 and 1910. During the summer of 1911 Mr. N. N. McDonald, of Edna, Tex., sent in some diseased plants for examination. The organism

[1] Halsted, B. D. Some fungous diseases of the sweet potato. New Jersey Agricultural Experiment Station, Bulletin 76, 1890.

[2] Saccardo, P. A. Sylloge Fungorum, v. 10, p. 173, 1892.

was found on the lower part of the vines and on the roots, the fruiting bodies appearing after a period of 8 or 10 days' incubation in a moist chamber. The same year Dr. F. A. Wolf, pathologist of the Alabama Agricultural Experiment Station, reported the occurrence of dry-rot in two counties of Alabama. In February, 1912, four potatoes were sent in from Indiana by Mr. Ludwig, then a student at Purdue University, and two of them had well-developed cases of the disease. In November, 1912, the disease was also found on vines of sweet potatoes received from Mr. Wallace O'Neal, of Aydlett, N. C. From time to time during the last two years, sweet potatoes have been collected from the Washington wholesale market, and on several occasions the dry-rot has been found. In most cases it was impossible, however, to get authentic data as to their source.

DESCRIPTION OF THE DISEASE.

Halsted in his description of this disease mentions its occurrence only on the roots. While it occurs more abundantly on the roots, it is not restricted to that part of the plant, but has been repeatedly found on the dead vines in the field and on the vines, petioles, and leaves of inoculated plants in the greenhouse.

The diseased potato is very much shrunken and wrinkled (Pl. I, figs. 1 and 2), finally becoming mummified. On the surface appear small pimples a millimeter or so in diameter lying close together and eventually covering the entire surface. If the epidermis is scraped slightly, the tissue beneath presents a coal-black carbonaceous appearance. Sections cut through this tissue show the numerous buried pycnidia[1] of the fungus causing the rot. The fungus enters the potato from the stem end and progresses slowly downward, eventually breaking down and destroying the cell walls. The contents of the cells become brownish, and the whole potato finally becomes dry and powdery. Stems thickly covered with pycnidia have been collected in the field, and pycnidia have been found very abundant on the stems of plants inoculated in the greenhouse. The stems of affected plants first turn yellow; and then, as they gradually die, slightly raised pimples appear, which finally break through the epidermis, showing the black pycnidia a millimeter or less in diameter lying close together. It was frequently found in the greenhouse that where the vines had grown very long a portion of the stem might be covered with pycnidia, while beyond the node where the vine had taken root the stem would be green and apparently healthy. Sections through the yellowed vine showed the mycelium of the fungus to be growing throughout the tissue of the stem.

[1] The fruiting stage on the root has been found only on the mummied potato. Fawcett in Bulletin 107 of the Florida Agricultural Experiment Station, 1911, makes a similar observation on the stem-rot of citrus fruits caused by a species of Phomopsis. He states that only rarely are spores formed on the decaying fruits in the grove, but are produced after the fruits become mummified upon the ground.

Pycnidia may also be found on the dead leaves[1] and petioles. They appear as small black dots scattered irregularly over the upper surface of the leaf.

PREVALENCE OF THE DISEASE.

THE DISEASE IN THE HOTBED.

Sprouts from diseased sweet potatoes which had been planted in sand in the greenhouse seemed at first to be perfectly healthy. It was found later, however, that the plants were diseased. This fact was established by cutting off the vines 8 or 10 inches long just below the node. After the vine had died back to the next node pycnidia would form abundantly on the dead surface. On these vines the stems were gradually killed back to the base, a yellowing of the stem preceding the formation of pycnidia.

The disease has been found on various occasions on the stems of plants in the old hotbeds which have been abandoned for the season. The pycnidia of the fungus do not develop on the living stem, but they appear on the dead stems as soon as the plants are killed by the crowded conditions of the hotbed or by other causes. Typical specimens of the disease were found in old hotbeds at Onley and Cape Charles, Va., in May, 1911. In June and July of the same year similar conditions were met with at Vineland, N. J. This would seem to suggest, as previously suspected, that the organism passes from the diseased potato used for seed into the young plants developed therefrom.

THE DISEASE IN THE FIELD.

It is difficult, if not impossible, to detect the presence of the disease in the field because of the fact that the fungus works so slowly and pycnidia do not form until the plant is in a languid or dying condition. This fact was brought out forcibly by some field inoculations made on the Potomac Flats during the spring of 1911. At harvest time plants which had been inoculated in June showed no evidence of being infected. It was thought hardly worth while to bring them into the laboratory after the examination made in the field. They were brought in, however, placed in damp chambers, and examined one or two weeks later. Out of 50 inoculated plants which showed no external evidence of the disease, 43 were found to be infected. In the fall of 1911, one or two weeks after digging, the organism was found on the dead vines in the field at Vineland, N. J. Since dry-rot is not discernible in the field at harvest time it is probably carried unsuspectingly into the storage house in the fall.

[1] The fungus isolated from the root, stem, and leaves has been compared culturally and morphologically and found to be identical. (See page 25.)

THE DISEASE IN THE STORAGE HOUSE.

The first signs of the disease appear in the storage house one month to six weeks after digging. The potatoes then show the characteristic dried, shriveled appearance starting at the stem end and gradually extending through the tissue. Later they become hard and mummified.

In view of the fact that the organism has been found on dead vines in the hotbed and in the field, and frequently in the storage house, there is little doubt that a complete cycle is formed by the fungus. The disease is undoubtedly carried on the slips into the field; thence into the storage house and subsequently on the "seed" to the hotbed.

THE FUNGUS.

Two forms of fructification of the dry-rot organism have been found—the pycnidial stage and the ascogenous stage. The pycnidial stage has been found frequently on the roots, stems, and leaves, but the ascogenous stage only in culture.

PYCNIDIAL STAGE.

In December, 1910, specimens of sweet potatoes were collected from the Washington market, which upon examination were found to be affected with the same disease that Ellis and Halsted described as *Phoma batatae*. The potatoes were considerably wrinkled at one end and somewhat powdery and browned within. Sections through the pycnidia showed that the organism present was not a typical Phoma, differing (1) in having two types of spores present (pycnospores and stylospores), (2) in chambering of the pycnidia, and (3) in the presence of a stroma. Additional specimens have been received and collected from New Jersey, Virginia, and elsewhere, showing similar macroscopic and microscopic characters.

The pycnidia found on the roots are at first buried, later breaking forth by a single ostiole. Longitudinal and tangential sections through the pycnidia show these to be irregularly chambered through lateral and basal projections of the wall (Pl. III, fig. 6). The pycnidia vary greatly in size, the side walls frequently coalescing, thus forming long continuous chambers. The ostiole varies considerably in length, in some cases being as long as the pycnidium itself.

The walls of the pycnidium are sclerotial to carbonaceous, the inner hyaline, the outer brown. Sections cut through the roots of all specimens examined show that the pycnidia are embedded in a well-developed stroma.

On the stems (Pl. I, fig. 3), petioles, and upper surface of the leaves the pycnidia, appearing as small, black specks, are at first covered but break forth more quickly than those on the roots.

They are numerous, usually separate, occasionally confluent, more or less globose, 60 to 130 μ by 60 to 110 μ, and have a short ostiole. A stroma is not found on the stems, petioles, or leaves.

The pycnospores are oblong to fusoid, 6 to 8 μ by 3 to 5 μ, continuous, hyaline, usually two guttulate; sometimes, however, with three oil droplets. They are borne on long hyaline, simple, continuous, filiform basidia, which are either straight or slightly curved (Pl. III, fig. 7).

In the same pycnidium with the pycnospores there may or may not be found another type of spore, the so-called stylospores. They are filiform, hook-shaped bodies, sometimes curved or rarely straight (Pl. III, fig. 5) 16 to 30 μ in length and continuous. The basidia are much shorter than those of the pycnospores (in most cases about half as long) and awl shaped (Pl. III, figs. 3 and 4). Stylospores or pycnospores may be found alone or together in the same pycnidium (Pl. II, figs. 1 and 2). They are commonly found on the roots and stems of the host, but have never been observed on the leaves or petioles. Repeated attempts to germinate the stylospores have been unsuccessful.

There has been considerable difference of opinion as to the nature and function of the stylospore. Saccardo [1] regarded them as conidiophores, "Basidia acicularia, demum hamata," in his description of the genus. Von Höhnel [2] made a new genus, Myxolibertella, to include those species having two kinds of spores, the oblong spores like those in the genus Myxosporium, and the filiform ones similar to those in the genus Libertella. He enrolled this new genus in the order Melanconiales but later placed it in the order Phomatales, genus Phomopsis. Bubák [3] and Diedicke [4] both considered these filiform bodies as spores. In an extensive treatise of the genus, Diedicke for convenience designates the Phomalike spore by the Greek letter alpha (α) and the long, filiform, hook-shaped spore by beta (β). Reddick [5] regards these spores in the organism causing dead-arm of the grape as paraphyses, placing the organism in the genus Fusicoccum, while Shear [6] regards these same bodies as spores and designates them as scolecospores. We have followed Nitschke's [7] terminology, using "stylospore" to designate the hook-shaped spore.

[1] Saccardo, P. A. Sylloge Fungorum, v. 18, p. 264, 1906.

[2] Höhnel, Franz von. Mycologische Fragmente. Annales Mycologici, Jahrg. 1, p. 526, 1903.

[3] Bubák, Franz. Vierter Beitrag zur Pilzflora von Tirol. Österreichische Botanische Zeitschrift, Jahrg. 55, p. 78, 1905.

[4] Diedicke, H. Die Gattung Phomopsis. Annales Mycologici, Jahrg. 9, p. 8–35, pl. 1–3, 1911.

[5] Reddick, Donald. Necrosis of the grape vine. New York Cornell Agricultural Experiment Station, Bulletin 263, p. 331, 1909.

[6] Shear, C. L. The ascogenous form of the fungus causing dead-arm of the grape. Phytopathology, v. 1, p. 116–119, 5 fig., 1911.

[7] Nitschke, Theodor. Pyrenomycetes Germanici, p. 240 [1869].

ASCOGENOUS STAGE.

The ascogenous stage has not been found on the host itself, although it has been obtained in culture. Specimens of sweet potatoes affected with dry-rot and vines covered with pycnidia were collected in the fall of 1911, and wintered outdoors on the ground in a wire cage in the hope of obtaining the perfect stage on the host. When these were examined the following spring no perithecia were found.

The organism was isolated in plates from sweet potatoes collected at the Washington market in December, 1910, and transfers made to beef agar and later to corn-meal flasks. These flasks were not examined until March 8, 1911, when two were found to contain mature asci. The other two contained only pycnospores and stylospores. It was at once suspected that this might be the ascogenous form of the dry-rot organism.

On May 22, 1911, transfers from one of the flasks containing asci were made to two other corn-meal flasks. When these flasks were examined on July 7 no asci were present, but pycnospores and stylospores were abundant. On October 17 both flasks were found to contain mature asci and ascospores. Poured beef-agar plates were made from one of the flasks containing asci. Two asci were located in the plates and ringed with india ink. They were kept under close observation for one or two days or until they had germinated and it was evident that the colonies could be transferred to beef-agar tubes without any foreign spores, mycelia, etc., accompanying them. Usually about two days were required to accomplish this, the plates being kept in an ice box during the night. Transfers were made from each tube on August 14 to two corn-meal flasks (four in all). In about five weeks asci were abundant in all of these flasks, but their formation was preceded by the production of pycnospores and stylospores.

On October 9 additional poured plates were made of asci from one flask of August 14. Eight asci were isolated and transferred to beef-agar tubes. Four corn-meal flasks were later inoculated from each tube (32 flasks in all). On October 21 eight flasks were examined, and all contained pycnospores and stylospores but no asci. When examined on November 2 asci were found in 22 flasks, and on November 6 they were present in all.

On October 31 poured plates were made from a flask of August 14 containing asci and 15 isolations made. Subsequently, transfers were made to as many mutton-agar tubes. On November 7 two corn-meal flasks and two rolled-oat flasks were inoculated from each tube (making in all 30 corn-meal and 30 rolled-oat flasks). On November 8 two tubes of sweet-potato cylinders were inoculated from each isolation (30 in all), and on the following day an equal

number of tubes of string beans were inoculated. On November 20 pycnospores and stylospores were abundant in all corn-meal flasks, and asci were found in all of them on December 15. On January 2, 1912, asci were found in all rolled-oat flasks and on the following day sparingly in tubes of sweet-potato cylinders, but none on string beans.

On December 19, 1911, poured plates were made from one of the corn-meal flasks of October 9, and 11 asci isolated and transferred to beef-agar tubes on December 22. Four days later two corn-meal flasks were inoculated from each tube (22 in all). On January 5, 1912, pycnospores and stylospores were found in all flasks examined. On February 1 all these flasks but three contained asci, and on February 6 asci were found in the three remaining flasks, which did not contain them on February 1.

On January 8 eight flasks each of wheat, oats, rye, and barley (32 in all) and nine tubes of sweet-potato stems were inoculated with the mycelia in the tubes from which the preceding set of experiments was made.[1] In a week to ten days following, pycnospores and stylospores were abundant. Asci were first observed in a flask of rye on February 1. On February 6 all the flasks contained asci and three tubes of the sweet-potato stems.

In studying the ascogenous stage of the dry-rot organism 36 asci in all have been isolated. When transfers were made from these strains to the proper medium, such as corn meal, perithecia were developed again in about 5 to 6 weeks. It is also interesting to note that on corn-meal flasks, and on other culture media where asci are produced, the pycnidial stage is developed first in 6 to 10 days. Preceding the development of the perithecia the pycnospores germinate and for the most part are not present when the asci are formed, but this is not an invariable rule.

Preceding the formation of asci, numerous club-shaped bodies (Pl. III, fig. 10) resembling the straight or slightly curved stylospores are formed. These are usually shorter than the typically hooked stylospore (Pl. III, fig. 5) and somewhat broader at one end or sometimes slightly swollen in the center, usually containing a number of oil droplets. In some instances these bodies assume the shape of immature asci, although they are never so large. Sections made through the stroma have never shown them to be present in the perithecia, but only in the pycnidial chambers. Diedicke describes the β spores (stylospores) in young pycnidia of *Phomopsis casuarinae* as needle or rod shaped, almost straight, rigid, sharply outlined, with a length of 13 to 16 μ. In old pycnidia these had

[1] While the cultures from which these transfers were made were 17 days old a careful examination showed that only mycelia were present in the tubes.

assumed their characteristic bending and were usually over 20 μ long. In our cultures we have never observed that these club-shaped bodies assumed the characteristic bending and longer length upon aging as mentioned by Diedicke. They have been found only in the ascogenous strain, but are regarded of the same nature as the hooked-shaped bodies, the difference in form being due to a difference in the two strains. The typically hooked-shaped stylospore is also present, but not as abundant as the club-shaped bodies.

Apparently two strains have been obtained, one which does not form perithecia in cultures (at least under ordinary conditions) and another which produces perithecia readily. Culturally and morphologically the pycnospores and stylospores of the two strains are the same when compared on various kinds of media.

The perithecia are formed in a Valsalike stroma (Pl. IV, fig. 2) which is ashy gray within, carbonaceous without, with many exserted, subcylindrical beaks from one-half to 3 mm. in length (Pl. IV, fig. 1). They are subglobose (Pl. IV, fig. 3) 120 to 370 μ in diameter, and the majority of the stromata contain from 9 to 25 perithecia. It is seen from a tangential section that the perithecia are formed in the same stroma with the pycnidia, frequently in a ring inside but separate from the pycnidial chambers. There is often a considerable variation from this type, as would naturally be expected in artificial cultures. Occasionally there is a tendency to form a double row of perithecia, some being more or less superimposed or irregularly arranged in the stroma. In no instance have asci been found in the pycnidia or pycnospores and stylospores in the perithecia.

The asci are clavate to cylindrical in shape, sessile, 8 spored, 23 to 38 μ by 7 to 12 μ (Pl. IV, fig. 4). The spores are subelliptical, obtuse at both ends, hyaline, one septate [1] slightly constricted at the center, 2 to 4 guttulate and 8 to 12 μ by 4 to 6 μ. The spores are usually arranged in two rows but occasionally in a single row and then obliquely. No paraphyses are present. The ascospores germinate in 3 to 5 hours at room temperature in hanging drop cultures made in distilled water or beef bouillon, the germ tubes usually coming from the side of the spore (Pl. IV, fig. 5) and growing very rapidly thereafter.

That this form represents the perfect stage in the life history of the dry-rot of sweet potatoes is based on the following facts: (1) The ascospore stage and the pycnidial stage were both derived from the same original isolation; (2) when a single ascus is isolated and

[1] On account of the presence of large oil droplets some difficulty was at first experienced in determining whether the ascospores were one or two celled. The septum was readily demonstrated, however, by making a mount of the spores in a solution of salicylic acid. The oil droplets soon disappeared and the presence of the septum became evident.

transferred to a corn-meal flask, a pycnidial stage is developed in 6 to 10 days which compares in every respect with the original strain; (3) the large numbers of asci isolated, which in all cases gave the same type of asci preceded by the pycnidial stage; and (4) successful infection obtained through inoculation from ascospore strain.

SYNONYMY.

After a careful study of the organism, it is evident that the imperfect form can not be referred to the genus Phoma, as was done by Ellis and Halsted, but that it probably belongs to the form genus Phomopsis. This genus is made up largely of forms which were originally classed as species of Phoma. Several characters, depending upon the author, have been emphasized as important in justifying this separation. Thus, Bubák[1] mentions two types of spores which evidently correspond to the pycnospores and what is here regarded as stylospores. He accordingly changed a number of fungi that were enrolled as Phoma to Phomopsis, among them *Phoma asparagi* Sacc. to *Phomopsis asparagi*. He furthermore calls attention to the fact that the spindle-shaped spores (pycnospores) and the septoria-like spores (stylospores) may occur together in the same pycnidium or separately. Similar observations have been made by Diedicke[2] who bases his claim for referring certain species of Phoma to Phomopsis to the following characters: (1) Chambered or locular pycnidia; (2) sclerotial stroma; (3) all forms having two types of spores, pycnospores and stylospores.

If Saccardo's classification is followed and the absence of stroma on the stem and leaves disregarded, the organism should be classed as a Dothiorella or a Fusicoccum, since Phomopsis is without a stroma. Following Diedicke's revision of the genus it is believed that the pycnidial form of this organism should properly be classed as a Phomopsis.

A comparison of type material of *Phoma batatae*, loaned by the New York Botanical Garden, with this species show that the two are identical macroscopically. A microscopic examination shows the pycnidia to be similar (1) in possessing a stroma, (2) in the chambering of the pycnidia, (3) in character of pycnidial walls, and (4) in having pycnospores and conidiophores which are of the same size and form. No stylospores were found in the type material.

The ascogenous form obtained in culture is a Diaporthe which in many species investigated by others has Phomopsis as the imperfect stage. The organism has been described as a new species, *Diaporthe*

[1] Bubák, Franz. Zweiter Beitrag zur Pilzflora von Montenegro. Bulletin de l'Herbier Boissier, s. 2, t. 6, p. 408, 1906.

[2] Diedicke, H. Die Gattung Phomopsis. Annales Mycologici, Jahrg. 9, p. 8–35, pl. 1–3, 1911.

batatatis.[1] Spegazzini [2] reports a species of Diaporthe (*D. ipomoeae*) occurring on *Ipomoea* sp. This species differs from *D. batatatis* in the following characters:

TABLE I.—*Comparison of Diaporthe ipomoeae with Diaporthe batatatis.*

Characters.	D. ipomoeae.	D. batatatis.
Asci	50 to 55×5 μ.	23 to 38×7 to 12 μ.
Spores	10 to 12×3 μ, uniseriate, oblique; not or only slightly constricted at septum; 1 to 2 oil droplets.	8 to 12×4 to 6 μ, biseriate occasionally uniseriate; distinctly constricted at septum; usually 4 oil droplets.

On account of these variations we do not consider the species identical.

PARASITISM OF THE ORGANISM.

INOCULATIONS IN THE GREENHOUSE.

Sweet potatoes do not thrive under greenhouse conditions as well as many other crops. The vines grew very luxuriantly, sending out long runners so that they overlapped, the checks and inoculated plants frequently running together, though the pots were some distance apart. As a result some of the checks were diseased. Potatoes were seldom formed, and when they were they were small.

The potatoes from which the sprouts were obtained for inoculation purposes were originally secured from the Washington market and selected as being free from disease so far as it was possible to judge from external appearances. They were then sterilized in formalin 1 to 200 for 20 minutes and rinsed in distilled water.

The original culture from which the inoculations were made was obtained in December, 1910, from some sweet potatoes collected at the Washington market.

On February 13, 1911, six inoculations were made on sweet-potato plants growing in 6-inch pots. An incision was made at the base of the stem with a sterile needle and mycelium from pure cultures inserted in the opening. Two plants were left as checks; on September 22 one plant was lifted. Pycnidia were numerous on the dying stems, but none were found on the roots. The roots were placed in a damp chamber and reexamined on October 19. The surface was then found to be covered with fruiting bodies containing both pycnospores and stylospores. Pure cultures of Phomopsis were secured by planting small bits of tissue from the interior of the potato in plates of beef agar. On October 19 the five remaining plants were lifted; pycnidia were numerous on the dying stems, but they were not found on the roots. The roots were placed in a damp

[1] Harter, L. L., and Field, E. C. Diaporthe, the ascogenous form of sweet-potato dry-rot. Phytopathology, v. 2, p. 121–124, 4 fig., 1912.

[2] Spegazzini, Carlo. Fungi Argentini novi v. Critici. Anales del Museo Nacional de Buenos Aires, t. 6 (s. 2, t. 3), p. 270, 1899.

chamber and examined on November 20. Four plants were found to have pycnidia with pycnospores and stylospores and one with pycnospores only. Both checks were infected. That the checks were also infected is to be explained by the fact that inoculated plants and checks were standing close together on the bench. It would thus be easy for the checks to become infected through actual contact with the diseased stems of the inoculated plants or by washing spores from one plant to another while watering.

On April 4, ten more inoculations were made on plants grown on a side bench of the greenhouse. These inoculations were made in the same way as those of February 13. The cultures used had been grown on beef agar at room temperature for five days and had not yet fruited. Two plants were left as checks. On December 4 the plants were lifted. The roots were poorly developed in each case. All of the inoculated plants and one check were infected.

On April 25, twenty more plants were inoculated. For this experiment cultures were grown in beef-bouillon tubes for seven days. There was an abundant mycelial growth in the bottom of the tubes, and in a few cases a pellicle had formed. One tube of these cultures was poured on the soil around each plant which had been transplanted to 9-inch pots. Four plants were left as checks. On September 22, three inoculated plants were lifted and all were found to be infected. On December 15 the remaining plants were lifted and 5 were found to be infected, making 8 infected plants out of 20 which were grown in the infected soil. None of the checks were infected.

Frequent reisolations have been made from inoculated plants and pure cultures of Phomopsis obtained, which were identical with the original strain.

INOCULATIONS FROM REISOLATIONS.

The organism was recovered on November 18, 1911, from infected plants inoculated in the greenhouse on April 4 by planting bits of the diseased tissue from stems on beef agar. On November 23 transfers were made from one of these colonies to sterilized string beans from which inoculations were made on December 4. The plants inoculated were obtained from potatoes which had been selected as being free from disease, disinfected in formalin (1 to 200) for 20 minutes and planted in sterilized sand. The slips were then transplanted in sterilized soil in 9-inch pots. Inoculations were made in ten plants by inserting mycelium and spores in a needle prick made at the base of the stem. Three plants were left for checks.

All the plants were lifted on May 7, 1912, and placed in moist chambers in the laboratory. On June 10 these were examined and pycnidia of Phomopsis found on all. Stylospores were found on nine plants. None of the checks were infected.

INOCULATIONS FROM ASCOSPORE STRAIN.

Asci from corn-meal flasks were isolated on October 31, 1911, in poured plates of beef agar and transfers made to mutton-agar tubes November 3. On November 9 fifteen young plants in 9-inch pots were inoculated by pricking the base of the stem and inserting mycelia (no pycnidia had been formed) with a sterile needle. Six plants were left for checks.

The slips were secured from potatoes selected as free from disease, sterilized with formalin (1 to 200) for 20 minutes and bedded in sterilized sand. They were then transplanted in pots, the soil of which had been steam sterilized.

On April 26, 1912, all the plants were lifted and placed in moist chambers. Subsequent examinations showed that none of the plants were infected.

On December 19, 1911, asci from corn-meal flasks were again isolated, and on December 22 transfers were made to beef-agar tubes. On December 26 seventeen more young plants were inoculated in the greenhouse in the same way and under similar conditions to the above series. Pycnospores were present in the culture. The plants were lifted on May 4, 1912, but it was found by later examinations that only one infection had taken place.

On August 2, 1912, fifty young plants on the Potomac Flats were inoculated at the base of the stem in the usual way from a 28-day-old culture of an ascospore strain on corn meal (pycnospores present). The plants were lifted on October 11 and placed in damp chambers. When examined on November 6 typical pycnidia of the conidial stage of the fungus had developed on nine of the plants. Isolations were made from three of these plants and the organism recovered.

On August 16 ten plants were inoculated on the Potomac Flats from a 14-day-old culture (pycnospores present) and fifteen plants from a 43-day-old culture (pycnospores and ascospores present) on corn-meal flasks. The plants were lifted on October 11 and placed in damp chambers. When examined on November 6 two plants inoculated with the 43-day-old culture were infected.

On August 26 ten more plants on the Potomac Flats were inoculated from a 14-day-old culture (pycnospores present) on corn-meal flasks. On October 11 they were lifted and placed in damp chambers. None of these plants became infected.

Fifty plants of the same age were pricked on August 2 at the base of the stem with a sterile needle and left as checks. These were also lifted on October 11 and placed in damp chambers, but none became infected.

Of the 85 plants inoculated on the Potomac Flats from cultures descended directly from asci, 11 developed typical cases of the dis-

ease. In each case there was no external evidence of disease when the plants were first lifted, as was found true in the inoculations from pycnospore strains. The disease did not become evident until after a period of incubation in the damp chambers.

INOCULATIONS IN THE FIELD.

The field inoculations were made from cultures descended from the original strain of this organism. Plants of the Commercial Jerseys were placed at our disposal on the Potomac Flats by Mr. W. R. Beattie, of the Office of Horticultural Investigations, and 25 were inoculated on June 12 and 25 more on June 19, 1911. Fifty checks in all were left at some distance from the inoculated plants. All the plants were inoculated at the base of the stem by making an incision with a sterile needle and inserting mycelium, spores, and a small bit of agar in the opening. The plants of June 12 were inoculated with a 6-day-old culture on beef agar and those of June 19 from a 9-day-old culture on beef agar.

The plants were lifted on October 20. The vines were cut off about 6 to 8 inches long and, with the smaller roots, were washed thoroughly in water and placed in moist chambers. In 10 days to two weeks pycnidia containing pycnospores and stylospores formed abundantly on the stems and vines and some on the roots. Of the 25 plants inoculated on June 12 twenty were infected. Of those inoculated on June 19 twenty-three were infected. None of the checks (50 in all) were diseased. The organism was reisolated from a number of these plants and when compared culturally was found to be identical with the original strain. The dry-rot organism evidently was not present in the field, since none of the 50 plants left as checks contracted the disease. In view of these successful inoculations it was possible to gain some knowledge of the character of this disease on the living plant under field conditions. So far as general external appearances were concerned these plants showed no symptoms of disease. They grew normally all summer and produced a crop equal to that of noninfected plants. This disease has never been observed elsewhere to cause any damage in the field. It is believed from these observations that it must be regarded principally as a storage trouble, but that infection of the plant takes place in the field or hotbed. The organism apparently grows very slowly, and pycnidia are never formed on the living growing parts of the plant.

REISOLATIONS FROM GREEN STEMS.

Although pycnidia have been found in abundance on the stems of plants inoculated in the greenhouse and on stems from abandoned seed beds, they have been observed only on those stems which were

beginning to turn brown. Planted plates were made from sections of green stems to determine if the fungus could enter the living tissue or gain an entrance only after the stem ceases to function as a living organism. In some cases the selected stems were partially dead and a few pycnidia were forming, the other end being normally green. Other stems were selected in which the vine between two nodes was dead and covered with pycnidia; beyond the nodes these stems were green and apparently healthy. All isolations were made from green stems. The stems were first washed in tap water, sterilized in $HgCl_2$ (1 to 1,000) for about 40 seconds, and then washed in sterile water. The epidermis was peeled off and sections cut and planted on beef-agar plates with the following results:

On October 27, 1911, plates were made from 6 stems, of which 4 gave pure cultures of Phomopsis.

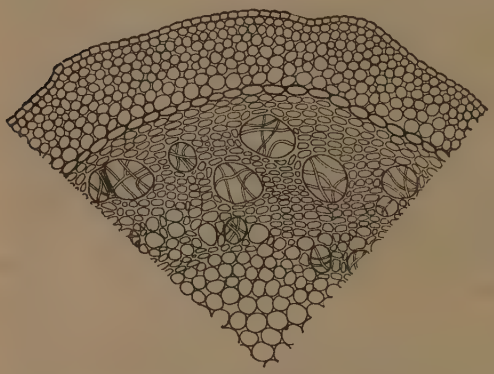


FIG. 1.—Section through a green sweet-potato stem, showing hyphæ in the bundles.

On November 27 plates were made from 11 stems, of which 4 gave pure cultures of Phomopsis; 3, no growth; a Fusarium was obtained from 1, while the remaining 3 developed bacterial colonies.

On July 25 and December 4 additional plates were made from stems of inoculated plants in the greenhouse and pure cultures of Phomopsis obtained. A record was not kept of the number of plants used. Isolations were again made March 1, 1912, from green stems and Phomopsis obtained (fig. 1).

SOME PHYSIOLOGICAL RELATIONS.

CULTURAL CHARACTERS.

Culture work was carried on quite extensively (1) in the hope of finding some medium which would produce the ascogenous stage and (2) to make a comparison of growth on some of the standard culture media.

INFLUENCE OF CULTURE MEDIA ON THE PRODUCTION OF ASCOSPORES.

The following media were used in an attempt to develop the ascogenous stage from the pycnospore strain: Sweet-potato cylin-

ders, sweet-potato agar, Irish-potato cylinders, corn meal, rice, string beans, beef agar, bouillon, sweet-potato stems, wheat, oats, rolled oats, barley, hulled barley, rye, buckwheat, mutton agar, and pork agar. A good growth was made on all of these media. Pycnospores formed on all the media and stylospores were produced in abundance on the following: sweet-potato cylinders, Irish-potato cylinders, corn meal, wheat, oats, rolled oats, barley, hulled barley, rye, and buckwheat. Pycnospores developed first in all the media, usually in about 5 days, the stylospores developing about two weeks later. Ascospores, however, were not produced.

An attempt was next made to see if acids or alkalis might prove effective in stimulating the fungus to produce ascospores. A definite amount of acid or alkali was added to sweet-potato agar, corn-meal flasks, and rice. A +10 medium of sweet-potato agar was first made up with the following acids: acetic, butyric, citric, formic, hydrochloric, malic, nitric, oxalic, phosphoric, and tartaric; a −10 NaOH was also used. Five tubes were inoculated for each test. Pycnospores and stylospores were produced in all the cultures, but no ascospores formed.

In making up the corn-meal flasks 10 grams of the meal were used, 60 c. c. of distilled water, and 1 c. c. of N/1 acid or the same amount of N/1 alkali were added to each flask. Besides the above-named acids and alkalis used in the experiments with sweet-potato agar, lactic acid, potassium hydrate, and sodium carbonate were added. Three flasks of each were used. A good growth was made in all the flasks except butyric acid, in which there was no growth, but no ascospores were formed.

The rice tubes were made up as follows: 0.75 gram of rice, 10 c. c. of distilled water, and 0.2 c. c. of N/1 acid or alkali added to each tube. The same acids and alkalis were used as those in the corn-meal flasks. Three tubes were inoculated for each test. No growth was made in the butyric-acid tubes. A good growth was made in all the other tubes, but no ascospores formed.

Thus far we have been unable to produce perithecia in culture from a pycnospore strain, although they are readily produced in corn meal, oats, barley, wheat, rye, and sweet-potato stems from an ascospore strain.

CHARACTER OF GROWTH IN DIFFERENT CULTURE MEDIA.

The behavior of an organism in certain culture media is sometimes so characteristic as to assist materially in its identification. Furthermore, other investigators may wish to repeat all or a part of the work recorded in this bulletin, and would in that case find such data of some value. For these reasons we have studied and recorded the growth of the dry-rot organism in nine of the more common culture media.

The media were not selected for any particular reason, except that they could readily be duplicated [1] by any one wishing to do so.

The inoculations were all made on the same afternoon (Feb. 14, 1912) from a 4-day-old culture (pycnospore strain) grown on beef agar. Five tubes (flasks in the case of corn meal) of each medium were inoculated and kept in the light in the laboratory, the temperature of which varied from 19° to 24° C. The cultures were examined frequently and careful notes made. The following records, given in number of days from the beginning of the experiment, briefly describe the character of the growth of the pycnidial stage of *Diaporthe batatatis* on the different culture media.

SWEET-POTATO CYLINDERS (825).

This medium was prepared by the usual method from sound, healthy potatoes and steamed on three consecutive days. Sweet-potato cylinders have been one of the best of media for the growth of this organism.

One day.—A brown discoloration extending about half an inch from the point of inoculation formed on the potato slant; hyphæ sparse (difficult to see) growing close to the medium and extending almost over the browned area.

Two days.—Brown discoloration extended over practically the whole surface of the slant; mycelium scant, becoming whitish.

Three days.—Good growth becoming heavier and fluffier; white dotlike masses of mycelium, becoming dark, covering surface of slant.

Five days.—A few fruiting bodies formed containing only pycnospores.

Six days.—Heavy, white growth becoming brown in places. Fruiting bodies not abundant. Only pycnospores present.

Seven, nine, ten, and twelve days.—Only pycnospores present.

Thirteen days.—Fruiting bodies abundant, covering surface of slant; pycnospores exuding from pycnidia in small, yellowish brown globules.

Fourteen and fifteen days.—Only pycnospores formed.

Sixteen days.—Typical stylospores formed, but not abundantly.

Thirty days.—Stylospores very abundant.

In this experiment pycnospores were formed in 5 days and stylospores in 16 days on sweet-potato cylinders. Records were made of the time required for the production of pycnospores and stylospores in transfers made on September 11 and September 28, 1911. On cultures made on September 11 pycnospores were formed in 4 days and stylospores in 26 days; in those made on September 28 pycnospores were formed in 5 days and stylospores in 23 days.

SWEET-POTATO AGAR (804).

One day.—Very thin growth started.

Two days.—Thin growth growing close to surface of media; small, white dotlike masses of mycelium scattered over growth.

Three days.—Good, heavy growth; white dots (fruiting bodies) beginning to turn dark.

Five days.—Fruiting bodies abundant; mycelial growth white; pycnospores present.

Six days.—Fruiting bodies very abundant; only pycnospores present.

Seven, nine, ten, and twelve days.—No apparent change in cultures.

Thirteen days.—Pycnospores oozing out from pycnidia in a yellowish brown slimy mass.

[1] The record numbers of the media are given, by means of which it is possible to obtain a complete description of the method by which it was prepared.

Fourteen, sixteen, nineteen, twenty-one, and twenty-two days.—No change.

Twenty-three days.—Stylospores present.

Sweet-potato agar has been the best of the agars for the growth and development of the pycnidia of this organism, and for that reason has been utilized as one of the standard media throughout the whole investigation. While stylospores appear somewhat irregularly on practically all media they occur with as much frequency on sweet-potato agar as on any other medium with perhaps the exception of corn meal.

IRISH-POTATO CYLINDERS (822).

This medium was prepared in the usual way from sound, healthy tubers and steamed on three consecutive days. Irish-potato cylinders have been a good medium for the growth of the dry-rot organism.

One day.—Brown discoloration, about half an inch in diameter, starting at point of inoculation. Hyphæ very thin, extending almost over the browned area.

Two days.—Brown discoloration extended practically over entire surface of slant. Mycelium scant, turning whitish.

Three days.—Mycelial growth close to surface of slant and covered with white dot-like masses of mycelia, some of the white dots becoming dark in color.

Five days.—Fruiting bodies abundant, containing pycnospores.

Six days.—Fruiting bodies more abundant.

Seven, nine, ten, and twelve days.—Only pycnospores present.

Thirteen days.—Spores exuding from pycnidia as small yellowish brown globules.

Fourteen, fifteen, and sixteen days.—Only pycnospores present.

Nineteen days.—Stylospores found.

Pycnospores were produced in the above cultures in 5 days, while the stylospores were not formed for 19 days.

CORN MEAL (838).

Corn meal in flasks was prepared in the usual way and autoclaved. Corn meal has been found from every standpoint the best of any of the media used. The organism always grew well and formed pycnidia and both pycnospores and stylospores.

One day.—No growth visible.

Two days.—Very small white growth, starting at point of inoculation.

Three days.—Thin growth, with pycnidia forming on surface.

Five days.—Good mycelial growth; fruiting bodies and pycnospores present. The formation of fruiting bodies gives a dark color to the colony, which is surrounded by a border of white, fluffy mycelium.

Six, seven, nine, ten, and twelve days.—Only pycnospores present.

Thirteen days.—Only pycnospores present. Spores exuding from pycnidia in small, yellowish brown globules.

Fourteen, fifteen, sixteen, and nineteen days.—Only pycnospores present.

Twenty-one days.—Stylospores found.

Pycnospores were formed in 5 days and stylospores in 21 days. Cultures made on September 11 on corn meal formed pycnospores in 4 days and stylospores in 26 days.

RICE (834).

One day.—No growth visible.

Two days.—No growth visible.

Three days.—Thin, white mycelial growth; fruiting bodies beginning to form.

Five days.—Fruiting bodies abundant, containing only pycnospores.

Six days.—Only pycnospores present.

Twenty-eight days.—Stylospores present, but not abundant.

Forty days.—Stylospores abundant.

Pycnospores were formed in 5 days; stylospores in 28 days.

PRUNE AGAR (774).

Prune agar has never been an especially good medium for the growth of this fungus.

One day.—Small growth started.

Two days.—Thin, white mycelial growth on surface of media.
Three days.—Fruiting bodies beginning to form.
Five days.—Few pycnidia present containing pycnospores.
No stylospores were formed in 33 days.

BEAN AGAR (773).

One day.—Hyphæ just starting at point of inoculation.
Two days.—Thin, white mycelial growth on the surface of the medium.
Three days.—Mycelial growth becoming heavier and a few white dotlike masses of hyphæ scattered over the surface.
Five days.—Fruiting bodies and pycnospores present, but not abundant.
Six days.—Very heavy, white mycelial growth. Fruiting bodies not abundant.
No stylospores formed in 33 days.

BEEF AGAR (813).

Beef agar has not been a good medium for the production of pycnidia.
One day.—Growth just starting.
Two days.—Thin, white, finely branched mycelial growth on surface penetrating the agar.
Three days.—Thin growth; no fruiting bodies formed.
Five days.—No fruiting bodies formed.
Six days.—Good, white mycelial growth; fruiting bodies and pycnospores present, but not abundant.
No stylospores formed in 33 days. An examination made after 51 days showed stylospores had formed very sparingly in one tube.

BOUILLON (824).

Bouillon has not been a good medium for the production of pycnidia and has been used in only a few instances.
One day.—Growth just starting at bottom of tube.
Two and three days.—Cottony, white growth at bottom of tubes.
Six days.—White, fluffy growth growing up toward the surface of the bouillon.
Thirteen days.—Pellicle formed in one tube.
Sixteen days.—Pellicle formed on all tubes. Pycnospores present.
No stylospores formed in 33 days.

The dates on which pycnospores and stylospores were found are given in the following table. The circles (○) and the crosses (+) indicate the dates on which pycnospores and stylospores, respectively, were found. The cultures were made on February 14, 1912.

TABLE II.—*Dates on which cultures were examined and the first appearance of pycnospores* (○) *and stylospores* (+).

Media used.	Date of examination.																									
	February.													March.												Apr. 5.
	15	16	17	19	20	21	23	24	26	27	28	29	1	4	6	7	8	9	11	13	15	18	22	25	
Sweet-potato cylinders (825)				○									+												
Sweet-potato agar (804)				○												+									
Irish-potato cylinders (822)				○									+												
Corn meal in flasks (838)				○											+										
Rice (834)				○																+					
Prune agar (774)				○																					
Bean agar (773)				○																					
Beef agar (813)					○																				+
Bouillon (824)													○												

It is seen from Table II that pycnospores form generally in about five days after inoculation and the stylospores somewhat later. The fruiting bodies appear at first as whitish, knotted masses (immature) on the substratum, but later turn black (mature). Pycnospores ooze out in a yellowish, viscous substance from the beak of the mature pycnidium. The fruiting bodies are much larger in culture and frequently provided with a long exserted beak.

It has been previously stated that the pycnidia occur on the root, stem, and leaves. No stylospores have been found in the pycnidia on the leaves, while they are commonly found in those on the stem and root. In view of this fact there was a possibility that they were different organisms. A comparison of the organism from root, stem, and leaf was made in cultures on different media. The results of this experiment are shown in Table III.

TABLE III.—*Comparison of growth on different media of isolations from the root, stem, and leaves.*

Media used.[1]	Date examined (in 1911).	Character of growth of organism from—		
		Root.	Stem.	Leaf.
String beans	Oct. 3	No pycnidia[2]	Pycnidia forming	Pycnidia forming.
	Oct. 7	Pycnidia (few)	Pycnidia (many)	Pycnidia (many).
	Oct. 21	Pycnospores present	Pycnospores present	Pycnospores present.
	Nov. 6[3]	do	do	Do.
Corn-meal flasks.	Oct. 3	Pycnidia in all flasks	Pycnidia in all flasks	Pycnidia in 3 out of 4 flasks.
	Oct. 7	Pycnidia numerous	Pycnidia numerous	Pycnidia numerous.
	Oct. 21	Pycnospores	Pycnospores	Pycnospores.
	Nov. 6	Pycnospores; stylospores.	Pycnospores; stylospores.	Pycnospores; stylospores.
Bouillon	Oct. 3	Growth cottony	Growth cottony	Growth cottony; pellicle forming.
	Oct. 7	do	do	Pellicle; a few pycnidia.
	Oct. 21	Pellicle (no pycnidia)	No pellicle	A few pycnospores.
	Nov. 6	Pellicle, pycnospores	Pellicle; no pycnidia	Pellicle; pycnospores.
Sweet-potato cylinders.	Oct. 3	Pycnidia numerous	Pycnidia forming	Pycnidia numerous.
	Oct. 7	do	Pycnidia numerous	Do.
	Oct. 21	Pycnospores; stylospores.	Pycnospores; stylospores.	Pycnospores; stylospores.
	Nov. 6	do	do	Do.
Beef agar	Oct. 3	Immature pycnidia	Immature pycnidia	Immature pycnidia.
	Oct. 7	Pycnidia (many)	Pycnidia (few)	Pycnidia (few).
	Oct. 21	Pycnospores (few)	Pycnospores (few)	Pycnidia (no spores).
	Nov. 21	Pycnospores	Pycnospores	Pycnospores.

[1] The cultures were made on Sept. 28, 1911.
[2] For the sake of brevity, the word "pycnidia" has been used in the sense of fruiting bodies.
[3] Examinations on various dates following Nov. 6 showed no change in the production of pycnospores or character of growth.

It seems evident from cultural characters that the organisms are identical. Numerous microscopical examinations and measurements of spores further justify this conclusion.

The mycelium has been carefully observed in different-aged cultures. On sweet-potato agar (841) the mycelia of 2-day-old cultures and cultures 3 weeks old differ somewhat in general character. In

either case it is very irregular in size, varying from 1.5 μ to 6 μ in diameter. From the first the cells are filled with granular bodies spherical or oblong in shape (fig. 2). While these bodies resemble oil globules, they do not color red when treated with a solution of Sudan III. Mycelia of cultures 3 weeks old have the same general appearance, but respond readily to the test for oils or fats. The mycelia of these cultures have, in addition, irregular, spherical, or oblong bodies, formed intercellularly, or as budlike processes from the mycelium (fig. 2, B, *a*) provided with one to several large oil droplets. Three-day-old cultures in prune agar developed numerous irregular, masslike bodies (fig. 3), the origin and function of which are not known.

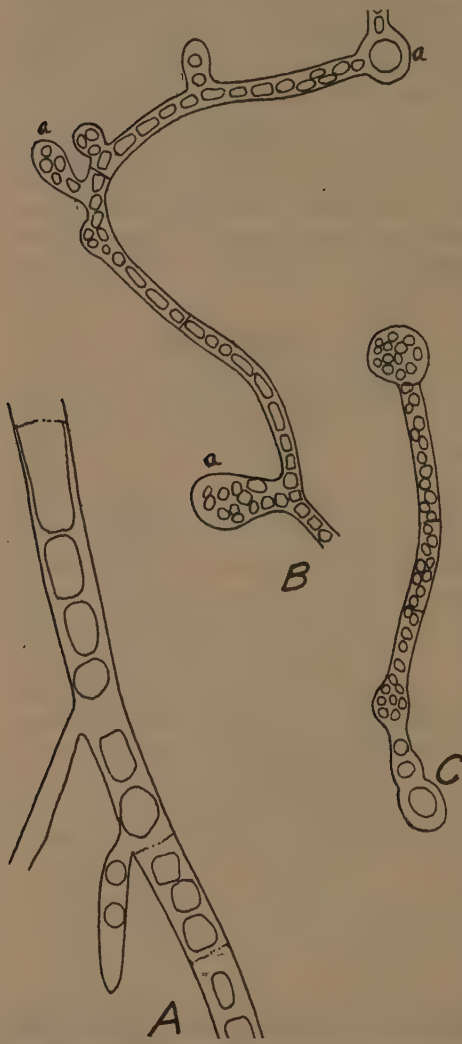


FIG. 2.—Hyphæ from a 2-day-old culture grown on sweet-potato agar.

INFLUENCE OF TEMPERATURE ON GROWTH.

The temperature at which this organism can grow and likewise produce fruit is important in view of the conditions under which the sweet-potato crop is stored. Contrary to the custom for most stored crops, the temperature for sweet potatoes is maintained considerably above freezing. A temperature of 27° to 32° C. (80° to 90° F.) for 10 days to 2 weeks is generally recommended for curing; then reduced to 10° to 15° C.

(50° to 59° F.) during the remainder of the storage period. However, no careful experiments to determine this fact have been carried out so far as the writers are aware. Generally a higher temperature is maintained in most storage houses, bringing it within the range for the best development of most fungi. Thermographs were placed in storage houses in New Jersey for several months during the winter of 1911 and 1912 and the records show that the temperatures ranged from 16° to 22° C. (61° to 72° F.) and occasionally higher. It was seldom that the temperatures got as low as 10° or 12° C. in these houses.[1] It would be desirable if a temperature could be maintained which could meet all the requirements so far as the storage of the sweet potato is concerned and at the same time prohibit or retard the growth of the organisms which cause decay.

FIG. 3.—Hyphæ from a 3-day-old culture grown on prune agar.

Cultures of the organism were made on sweet-potato agar (841) from a 5-day-old culture grown on sweet-potato agar, and three tubes were placed in each of 12 thermostats ranging in temperature from 0.5° to 38.6° C. and kept under observation for 18 days. One set of tubes was kept in the laboratory at room temperature as a check, it having been previously ascertained that this represented a good temperature for growth. The results of these experiments are recorded in Table IV.

Figure 4 shows that mycelial growth and the formation of fruiting bodies were greatly retarded at the lower temperatures. During the 18 days in which the experiment was conducted no growth occurred in Thermostats I and II (0.5 to 6.0° C.), or in XII (37 to 38.6° C.) after the first few days, during which a heavy aerial growth started at the point of inoculation. The mycelia attained its maximum growth in Thermostats X (23.8 to 27° C.) and XI (30.7 to 31.8° C.).

[1] In numerous instances where the relative humidity was determined by the sling psychrometer it was found to vary from 50 to 98 per cent. A relative humidity of 80 per cent was common. The relative high humidity, accompanied by a high temperature, provides optimum conditions for the dry-rot fungus.

TABLE IV.—*Records of growth in laboratory room and 12 thermostats maintained at different temperatures (° C.)*[1].

Date.	I. Ave., 1.09; Max., 1.8; Min., .5.	II. Ave., 4.2; Max., 6.0; Min., 3.3.	III. Ave., 7.5; Max., 9.2; Min., 6.0.	IV. Ave., 8.3; Max., 9.5; Min., 7.0.	V. Ave., 8.7; Max., 10.0; Min., 7.5.	VI. Ave., 12.3; Max., 14.0; Min., 11.2.	VII. Ave., 14.7; Max., 15.8; Min., 13.5.
Feb. 28	No growth.	No growth.	No growth.	No growth.	No growth.	No growth.	Very thin growth of but few threads of hyphæ; media slightly browned.
Feb. 29	do	do	do	do	do	do	Very thin growth; only a few strands of hyphæ arising from point of inoculation.
Mar. 1	do	do	do	do	do	Very sparse growth.	Very thin growth, consisting of a few branched hyphæ.
Mar. 2	do	do	do	do	Very sparse growth starting on one tube.	Sparse growth.	Thin growth, 3 cm. in diameter.
Mar. 4	do	do	do	Very sparse growth, a few millimeters in diameter.	Very sparse growth.	do	Fair growth, becoming white.
Mar. 5	do	do	do	Sparse growth, 1 to 2 cm. in diameter.	Sparse growth.	Growth becoming whitish.	Good white growth; no pycnidia.
Mar. 6	do	do	do	Thin growth.	Thin growth, 2 cm. in diameter.	Whitish growth not heavy, 3 to 4 cm. in diameter.	No pycnidia.
Mar. 7	do	do	do	do	Thin growth.	Good white growth.	Heavy white growth; no pycnidia.
Mar. 8	do	do	do	do	do	do	Do.
Mar. 9	do	do	do	do	do	do	Do.
Mar. 11	do	do	do	do	Growth becoming white.	do	Do.
Mar. 12	do	do	do	Becoming white.	do	Heavy white growth; no pycnidia.	Do.
Mar. 13	do	do	do	Good growth.	Good growth.	do	Do.
Mar. 14	do	do	Thin growth starting on one tube.	do	do	do	Do.
Mar. 15	do	do	do	do	do	do	Do.

[1] Temperature readings were made about 9.15 a. m. and 4.15 p. m.

TABLE IV.—*Records of growth in laboratory room and 12 thermostats maintained at different temperatures* (° *C.*)—Continued.

Date.	VIII. Ave., 16.2; Max., 17.0; Min., 14.5.	IX. Ave., 17.1; Max., 18.0; Min., 15.2.	Room.[1] Ave., 22.5; Max., 26.0; Min., 20.0.	X. Ave., 24.9; Max., 27.0; Min., 23.8.	XI. Ave., 31.4; Max., 31.8; Min., 30.7.	XII. Ave., 37.8; Max., 38.6; Min., 37.0.
Feb. 28	Very thin growth of but few threads of hyphæ; media slightly colored.	Thin growth, 1 cm. in diameter; brown discoloration of media.	Thin growth, 2 to 2.5 cm. in diameter; brown discoloration of media.	Thin growth, 2 to 5 cm. in diameter; brown discoloration of media.	Thin growth, 3 cm. in diameter; slight discoloration of media.	Heavy aerial growth at point of inoculation, not spreading; slight discoloration.
Feb. 29	Very thin growth.	Thin growth, 2 to 2.5 cm. in diameter.	Fair growth, not as good as in X or XI, 3 cm. in diameter.	Good white growth, 4 cm. in diameter; hyphæ slightly raised from media.	Growth covering whole slant; hyphæ slightly raised.	Growth not spreading.
Mar. 1	Thin growth, extending 2 to 3 cm. in diameter.	Growth becoming whitish, 3 to 4 cm. in diameter.	Heavy growth.	Heavy growth; pycnidia[2] starting to form.	Heavy growth; no pycnidia starting.	Do.
Mar. 2	Growth becoming whitish, no pycnidia.	Growth becoming heavy; no pycnidia.	Heavy growth; pycnidia forming more abundantly than in X.	Heavy growth; pycnidia forming in all tubes.	Heavy growth, becoming somewhat brownish; no pycnidia.	No further growth.
Mar. 4	Not as good as IX; no pycnidia.	No pycnidia	Pycnidia very abundant.	Pycnidia not as abundant as in room, but larger.	No pycnidia	Do.
Mar. 5	Good growth; no pycnidia.	do	do	do	do	Do.
Mar. 6	No pycnidia.	do	do	Pycnidia much larger but not as abundant as in room.	A few pycnidia beginning to form.	Do.
Mar. 7	do	do	do	do	Pycnidia forming on all tubes.	Do.
Mar. 8	Heavy white growth; no pycnidia.	do	do	do	Pycnidia not abundant.	Do.
Mar. 9	do	do	do	Beaks becoming abundant on pycnidia.	do	Do.
Mar. 11	do	One pycnidium formed on one tube.	do	do	do	Do.
Mar. 12	do	Pycnidia forming in all tubes.	do	do	do	Do.
Mar. 13	do	do	do	do	do	Do.
Mar. 14	do	do	do	do	do	Do.
Mar. 15	Pycnidia forming in two tubes.	do	do	do	do	Do.

[1] The cultures were placed on a table in the laboratory with northern exposure.
[2] For the sake of brevity, the word "pycnidia" has been used in this table in the sense of fruiting bodies.

Fruiting bodies were formed in Thermostat VIII (14.5 to 17° C.) at the end of 18 days; in IX (15.2 to 18° C.) at the end of 14 days; in room (20 to 26° C.) at the end of 5 days; in X (23.8 to 27° C.) at the end of 5 days; in XI (30.7 to 31.8° C.) at the end of 9 days. The optimum temperature for the formation of fruiting bodies appears to be between 20 to 27° C. Fruiting bodies were much more abundant but smaller in the tubes in the room than in Thermostat X, light probably having more influence on development than temperature.

The organism was not killed in Thermostats I and II (0.5 to 6.0° C.) and XII (37.0 to 38.6° C.), but growth was inhibited. Tubes from Thermostats I and II were removed on March 15 and placed in room temperature; those in XII were removed the day following. On March 20 these cultures were examined and a heavy mycelial growth

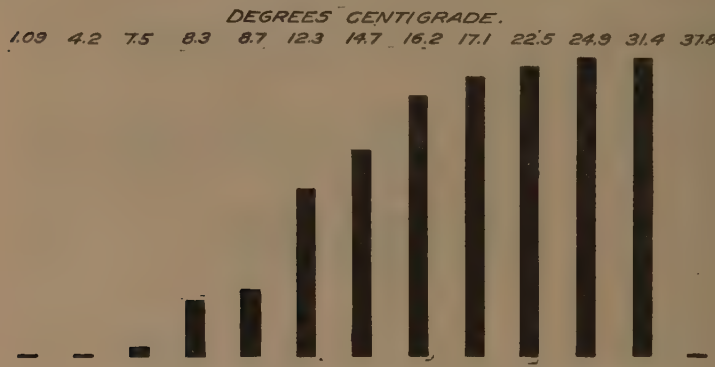


FIG. 4.—Graphic representation of growth on sweet-potato agar at different temperatures.

producing numerous fruiting bodies was found in all the tubes. Cultures removed from Thermostats IV and VI also fruited abundantly when brought into room temperature.

It should be remembered that these results are only approximate, no attempt having been made to establish absolute limits. There is considerable difference between 31.4° C., where growth is good, and 37.8° C., where none takes place. The exact temperature at which growth is prohibited can be established only by extensive experiments.

INFLUENCE OF LIGHT ON THE GROWTH AND FORMATION OF FRUITING BODIES.

Considerable difficulty was experienced at different stages of the work to induce the formation of fruiting bodies on beef agar. In some cases fruiting bodies would form abundantly, then again they were not produced. Usually the cultures were kept in a thermostat at an almost uniform temperature, but even then fruiting bodies

were in general only sparingly formed.[1] On the other hand, cultures on the same medium placed in parts of the laboratory exposed to light would develop numerous fruiting bodies in a comparatively short time. This naturally suggested the possibility that light was the important factor so far as the production of fruiting bodies was concerned. Tubes of slanted sweet-potato agar (841) were inoculated with the spores and mycelia of the organism on March 9, 1912. Four tubes were set on the laboratory table in the center of the room fully exposed to the light which was admitted from two large north windows. Four tubes were placed about a foot from one of the windows and directly exposed to the light, while four others were set in a tin crate and covered with two thicknesses of black cloth through which little or no light was admitted. The temperature at the middle of the laboratory varied from 18 to 24° C. and averaged about 22° C. Near the window the temperature was one or two degrees lower.

At the end of 48 hours little or no difference could be seen between the mycelial growth in the tubes exposed to light and those in the dark. In fact, the tubes in the dark gave as vigorous a growth of mycelia even to the end of the experiment as those exposed to light. There was, however, a very marked difference in the formation of fruiting bodies. Three days after the tubes were inoculated numerous white specks, which later proved to be inceptive fruiting bodies, appeared in the tubes exposed to light, while they were completely absent in those kept in darkness. One day later these bodies began to darken and pycnospores were found in them. On March 15 a few large scattered fruiting bodies were found in tubes in the dark and a few spores. On March 16 fruiting bodies were found abundantly over the whole slant of tubes exposed to the light. After March 18 no more fruiting bodies formed in any of the tubes. In darkness the fruiting bodies varied from 8 to 25 in each tube, while in those exposed to the light they were very numerous, perhaps several hundred in each. In the dark the fruiting bodies were 2 to 3 mm. in diameter, which is many times larger than those on the same medium in the light or on the host under natural conditions. The fruiting bodies formed in darkness, while apparently well developed, were frequently sterile, and at no time were spores exuded. On the other hand, in the light the spores were exuded in great abundance as large, viscous, yellow globules.

It has been repeatedly observed that cultures grown in the dark have much larger fruiting bodies but are fewer in number than those grown in the light.[2]

[1] Thermostats run at different temperatures gave similar results.

[2] Mycelial growth was found to be more vigorous in cultures grown in thermostats X and XI (Table IV), where the temperatures (23.8 to 31.8° C.) ran higher than in the room. The formation of fruiting bodies, especially in Thermostat XI, was much less abundant, however, than in the room. If these had been grown in the light instead of in the dark the fruiting bodies might have been much more abundant than in cultures grown at room temperature.

GERMINATION OF PYCNOSPORES.

Pycnospores germinate readily in hanging-drop water cultures at room temperature in about 3½ hours. There is generally only one germ tube (Pl. III, figs. 8 and 9), occasionally two from each spore, and these may come from either the side or end.

INFLUENCE OF TEMPERATURE ON THE GERMINATION OF PYCNOSPORES.

The temperature at which the spores will germinate is of some importance for a crop which is handled like sweet potatoes. For several months they are kept in storage houses, and unlike the Irish potato, cabbage, and most other crops, must be stored at a rather warm temperature.

To determine the limiting temperatures for germination of the pycnospores, hanging-drop cultures were made in Van Tieghem cells in tap water from 6-day-old cultures grown on sweet-potato agar under sterile conditions. These were placed in Thermostats I, V, X, XI, and XII, and one set left in room temperature as a check. Examinations were made at the end of 17, 41, 65, and 113 hours.

TABLE V.—*Limiting temperature for the germination of pycnospores.*

Thermostat.	Temperatures.	Time in hours.			
		17	41	65	113
	°C.				
I	0.8 to 1.8	No germination.	No germination.	No germination.	No germination.
V	8.5 to 9.0	do	Slight germination.		
Room	20.0 to 23.0	Some germination.	Good germination.		
X	24.3 to 24.7	Good germination.			
XI	31.2 to 31.5	do			
XII	37.5 to 38.1	No germination.	No germination.	No germination.	No germination.

Table V shows that the optimum temperature for growth lies somewhere near or between 24.5° and 31.5° C. Careful observations showed no difference in the germination of the spores at these two temperatures. At room temperature, which varied from 20° to 23° C., germination was somewhat slower at the outset, but at the end of 41 hours was very good. At the end of 41 hours only a small percentage of the spores germinated at temperatures ranging from 8.5° to 9.0° C., and these made a feeble growth thereafter. The minimum temperature for growth lies somewhere between 9° and 1.8° C. At the latter temperature no germination took place at the

end of 113 hours. The maximum temperature for spore germination is somewhere between 31.5° and 38.1° C., but no germination took place at the end of 113 hours at temperatures varying from 37.5° to 38.1° C.

The hanging-drop cultures placed in Thermostats I and XII, after being removed therefrom, were kept at room temperatures for 48 hours. It is interesting to note that at the end of that time the spores from Thermostat XII did not germinate and evidently had been killed by the higher temperature. On the contrary, the spores from Thermostat I germinated at first somewhat feebly but rather vigorously later.

It is apparent that the pycnospores of the dry-rot fungus will germinate at the lower temperature generally recommended for the storage houses (about 10° C.). As the temperature rises above that point the spores germinate more readily, at least up to a temperature of 31.5°, but is completely prevented at 38° C. Either of these temperatures would be too high to store the sweet potato successfully.

VIABILITY OF PYCNOSPORES.

The viability of the pycnospores in cultures has been determined for only three media. The experiment was undertaken at the outset to determine the length of time the spores would live in cultures, it being presumed that their viability would be retained equally long on all media. String beans, sweet-potato cylinders, and beef agar were used for the experiment. Hanging-drop cultures were made in tap water in Van Tieghem cells. Tap water was used for the reason that it was previously found that spores would not germinate readily in distilled water.

Cultures were made on September 28, 1911, on string beans, sweet-potato cylinders, and beef agar, and hanging drops from these cultures on February 2, 1912. Four hanging-drop cultures were made from each medium on each of three consecutive days and no germination of spores from string beans and beef agar took place at the end of 24 hours. The spores from sweet-potato cylinders, on the other hand, germinated in a few hours at room temperature.

The whole series of tubes was examined on March 7, and it was found that the spores had completely disappeared. As a general rule the pycnospores do not endure in cultures very many months. Repeated search has failed to find them in old cultures. Whether or not they germinate and fail to produce pycnidia again is not certain. It is believed, however, that they disintegrate. On the stems the organism will live for six months and germinate readily at the end of that time. Pycnidia and spores were found on vines wintered

out of doors from October until the following June. At the end of that time pure cultures were secured, showing that the organism is probably carried through the winter by means of the pycnospores.

THERMAL DEATH POINT.

It has already been noted that cultures of this organism on sweet-potato agar, subjected to the average temperatures of 1.09°, 4.2°, and 37.8° C. for 18 days, made no growth. When these cultures were removed from the thermostat and placed in the room at a temperature varying from 18° to 24° C. a vigorous growth took place, which showed that the organism had not been killed during this period of incubation. Experiments were then made to ascertain how high a temperature would be required to kill pycnospores.

Beef-bouillon tubes[1] were inoculated with spores which were exuding from the pycnidia grown on sweet-potato agar. The cultures were immersed in a water bath at the desired temperature for 10 minutes,[2] the water being constantly stirred by a paddle operated automatically; the tubes were then removed and left in room temperature 1 to 2 weeks.

It was found that no growth occurred in any of the tubes exposed to a temperature of 49° C.[3] or higher. Of the cultures 80 per cent grew when exposed to a temperature of 46° C.; 40 per cent at 47° and 48° C.[4] While these results are only approximate, the thermal death point of the pycnospores evidently lies between 48° and 49° C. under these conditions.

TOXICITY OF COPPER SULPHATE TO PYCNOSPORES.

The toxicity of $CuSO_4$ was determined by means of hanging-drop cultures in Van Tieghem cells. In view of the fact that the spores did not germinate well in distilled water, the copper-sulphate solutions were made with tap water. The cultures were made from the spores that were oozing from pycnidia grown on sweet-potato agar. They were then placed in a thermostat run at a temperature of 25° C. Check cultures were made in tap water in which the spores germinated freely and made a good growth in 24 hours.

The results of the experiment showed that at the end of 48 hours the pycnospores would not germinate in a 1 to 100,000 $CuSO_4$ solution, but in a solution of 1 to 120,000 short germ tubes were pushed out in probably 5 per cent of the spores in 24 hours. When the

[1] Tubes of uniform size and thickness of glass were selected for all these tests.

[2] The temperature of the water during the period the tubes were exposed did not vary more than one-half of 1 degree.

[3] Five inoculated tubes were exposed to a temperature of 49° C. on three different occasions.

[4] Twenty inoculated tubes were exposed to a temperature of 47° and 48° C. on four different occasions.

solution was diluted 1 to 160,000 the percentage of germinating spores perceptibly increased and the germ tubes were much longer. Even at this concentration germination and growth were materially reduced. From these results it would seem that 1 to 120,000 is about the limit of toleration, since there was little or no increase in germination or growth at the end of one day more.

TABLE VI.—*Germination of pycnospores in tap water and in solutions of copper sulphate ($CuSO_4$).*

Date, March, 1912.	Length of time (hours).	Tap water.	Strength of solution.										
			1 to 5,000.	1 to 6,000.	1 to 10,000.	1 to 12,000.	1 to 20,000.	1 to 40,000.	1 to 80,000.	1 to 100,000.	1 to 120,000.	1 to 160,000.	1 to 200,000.
8	24	Germination	.....	(1)	.....	(1)	.....	.....	.....	.....	.....	.....	.....
9	24	.....do	(1)	.....	(1)	.....	(1)	.....	.....	.....	.....	.....	.....
10	24	Good germination	.....	.....	.....	.....	.....	(1)	(1)	.....	.....	(2)	.....
13	24	.....do	.....	.....	.....	.....	.....	.....	(1)	.....	.....	(2)	(2)
14	24	.....do	.....	.....	.....	.....	.....	.....	.....	.....	.....	(2)	.....
15	24	.....do	.....	.....	.....	.....	.....	.....	.....	(1)	.....	.....	.....
16	48	.....do	.....	.....	.....	.....	.....	.....	.....	(1)	(2)	.....	.....
18	24	.....do	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
19	24	.....do	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

[1] No germination. [2] Small percentage just starting to germinate.

DISSEMINATION OF THE DISEASE.

The organism causing dry-rot of the sweet potato is undoubtedly distributed with the potato itself, and not to any extent by such agencies as the wind, insects, etc., as is the case with many other fungi. Probably the most conspicuous source of distribution is the potatoes selected for seed. A slight infection of the potato by this fungus is likely to be overlooked by anyone not familiar with the disease. Diseased potatoes when used for seed can be expected to give diseased slips, which in turn carry the organism to the field.

We have been able to prove in the greenhouse the transfer of the disease from the potato to the slip. Potatoes were selected which were known to have the disease and put in sand to sprout in one of the houses where no other sweet potatoes were growing, thus eliminating the possibility of infection from any other source. They sprouted freely and produced numerous plants which had the characteristic pycnidia on the vines and leaves. There is little possibility of detecting the disease on the slips in the seed bed before or even at planting time. The affected plants show no signs of the disease. The organism does not appear to be a destructive parasite, the host and parasite living in apparent harmony, at least for a considerable time. As soon, however, as the host is

weakened from any cause the parasite becomes evident by the formation of pycnidia and pycnospores. Hence, many diseased plants are likely to be carried undetected to the field, where the organism during the course of the summer may invade the roots. These roots are carried to the storage house, no one suspecting from their appearance that they are diseased. In the storage houses under favorable conditions, preferably warm and moist weather, the organism grows rather rapidly, invading and destroying all parts of the tissue of the potato, producing a dried, wrinkled condition. Potatoes infected in this manner are unsalable, and in the winter when they are picked over are discarded. These discards are disposed of in various ways, depending upon the available opportunities. Some people feed them to chickens, others to the pigs, and sometimes to cattle. They may or may not be cooked before feeding to stock. They generally are not. Those who do not feed them to stock throw them out on a pile and in the spring haul them onto the fields. Hundreds of bushels are disposed of in this manner. The dry-rot organism has been found in many instances in these piles.

METHODS OF CONTROL.

No experiments have been conducted in controlling this disease, but the methods to be employed would seem to be self-evident. Precaution should be the first consideration, and the following suggestions are made which if practiced it is believed will greatly reduce the loss from this disease. Great care should be exercised in the selection of potatoes for seed. When potatoes are selected for seed in the fall they should be examined again in the spring and those showing the slightest symptoms of the disease should be discarded. The seed bed should be made preferably of new soil, so that there is less possibility of the spores being present, or if old soil is used it should be sterilized, if possible, by steam at a temperature of 100° C. for one hour. In the absence of steam sterilization the beds may be soaked with formalin (40 per cent), one part to 200 parts of water. If the latter method is followed the soil should be treated 10 days to two weeks before it is to be used and stirred occasionally to facilitate the evaporation of the formalin. As an additional precaution, the potatoes before bedding should be disinfected with a solution of formalin (1 part of 40 per cent formalin to 200 parts water) or corrosive sublimate (1 part corrosive sublimate to 1,000 parts water) for 20 minutes, then rinsed in water and dried.

When it is desirable to feed the discarded potatoes to stock, they should be cooked. They should not be thrown on the manure pile to compost and then hauled to the field.

SUMMARY.

(1) The ascogenous form of the organism causing dry-rot of sweet potatoes is a Diaporthe and has been given the species name *batatatis* by the writers.

(2) The pycnidial stage was first described by Ellis and Halsted as *Phoma batatae*, but the imperfect stage has here been considered as belonging to the form genus Phomopsis.

(3) The disease is principally a storage trouble. It is characterized by a drying and shriveling of the potato, on the surface of which appear many small pimples, the pycnidia, lying close together and eventually covering the entire surface.

(4) The disease also occurs in the hotbed and in the field. The organism is not a vigorous parasite and consequently under ordinary conditions does not become evident until some time after harvesting. Diseased vines have been collected in abandoned hotbeds.

(5) The pycnidial stage develops from the ascospore in 6 to 10 days.

(6) Ascospores are formed from the ascogenous strain in 4 to 6 weeks on cereals, but they are always preceded by the formation of pycnidia.

(7) Both stages were derived from the same isolation.

(8) Inoculations with pure cultures of the pycnidial stage both in the greenhouse and in the field gave successful infections.

(9) Successful infections were also obtained from reisolations.

(10) Inoculations on the Potomac Flats with pure cultures of the ascogenous strain gave successful infections.

(11) Isolations of the organism were secured in planted plates from green stems of infected plants. Pycnidia, however, form only on the dead or moribund tissue.

(12) In the greenhouse, pycnidia occur on the leaves, stems, and roots of inoculated plants.

(13) The ascogenous stage fruits well on all cereals tried and sparingly on sweet-potato cylinders and sweet-potato stems.

(14) Light and high temperatures stimulate the production of the fruiting bodies in culture.

(15) The disease is disseminated principally by means of the "seed" and by the use of decayed potatoes for manure.

(16) Diseased potatoes should be cooked before feeding to stock. They should never be scattered on the fields as a fertilizer.

(17) Seed beds should be sterilized and potatoes to be used for seed carefully selected.

DESCRIPTION OF PLATES.

PLATE I. Fig. 1.—Sweet potato wrinkled by the dry-rot organism. Fig. 2.—Sweet potato, showing the darkened fruiting bodies of the dry-rot organism. Fig. 3.—Sweet-potato stem, slightly enlarged, showing the pycnidia of the dry-rot organism.

PLATE II. Fig. 1.—Microphotograph of a longitudinal section of a culture on sweet-potato agar, showing pycnidia and stroma. Fig. 2.—A single pycnidium from figure 1 more highly magnified, showing the presence of pycnospores and stylospores.

PLATE III. Pycnidial stage of *Diaporthe batatatis* Harter and Field. Fig. 1—Longitudinal section through a pycnidium containing pycnospores. Fig. 2.—Tangential section from sweet potato, showing the arrangement of the pycnidia in the stroma. Figs. 3, 4, and 5.—Stylospores and conidiophores. Fig. 6.—Tangential section from sweet potato, showing the chambering of the pycnidium which contains stylospores. Fig. 7.—Pycnospores and conidiophores. Figs. 8 and 9.—Germination of pycnospores. Fig. 10.—Club-shaped stylospores found in connection with ascospore strain.

PLATE IV. Ascogenous stage of *Diaporthe batatatis* Harter and Field. Fig. 1.—Longitudinal section through a fruiting body, showing elongated beaks. Fig. 2.—Tangential section through a stroma, showing a Valsalike arrangement of the perithecia. Fig. 3.—Tangential section through a perithecium, showing asci and ascospores. Fig. 4.—Asci and ascospores. Fig. 5.—Germinating ascospores.

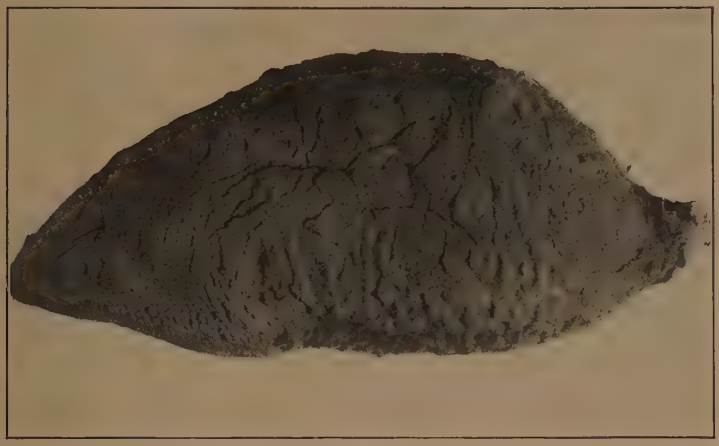


FIG. 1.—SWEET POTATO WRINKLED BY THE DRY-ROT ORGANISM.

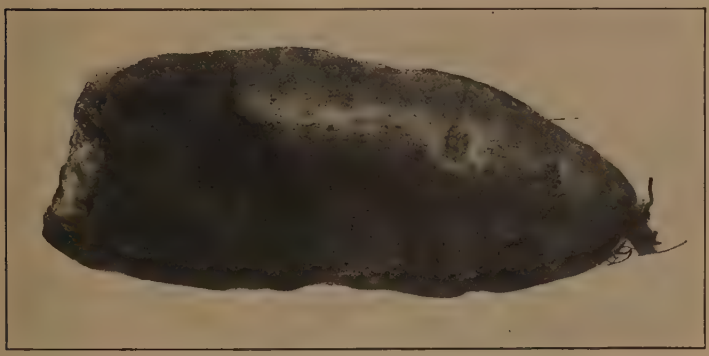


FIG. 2.—SWEET POTATO, SHOWING THE PYCNIDIA.

FIG. 3.—SWEET-POTATO STEM, SLIGHTLY ENLARGED, SHOWING THE PYCNIDIA.

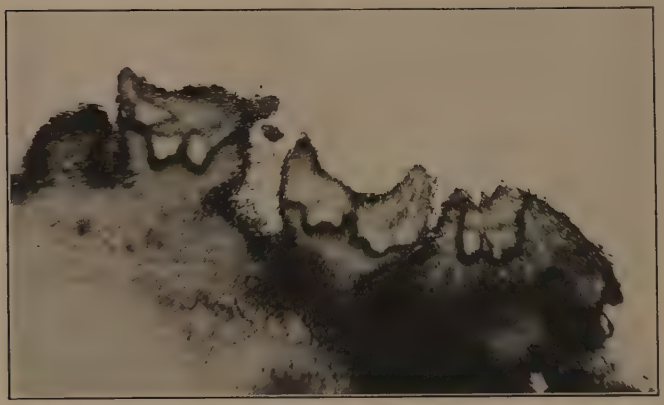


FIG. 1.—LONGITUDINAL SECTION THROUGH A GROUP OF PYCNIDIA GROWN ON SWEET-POTATO AGAR.

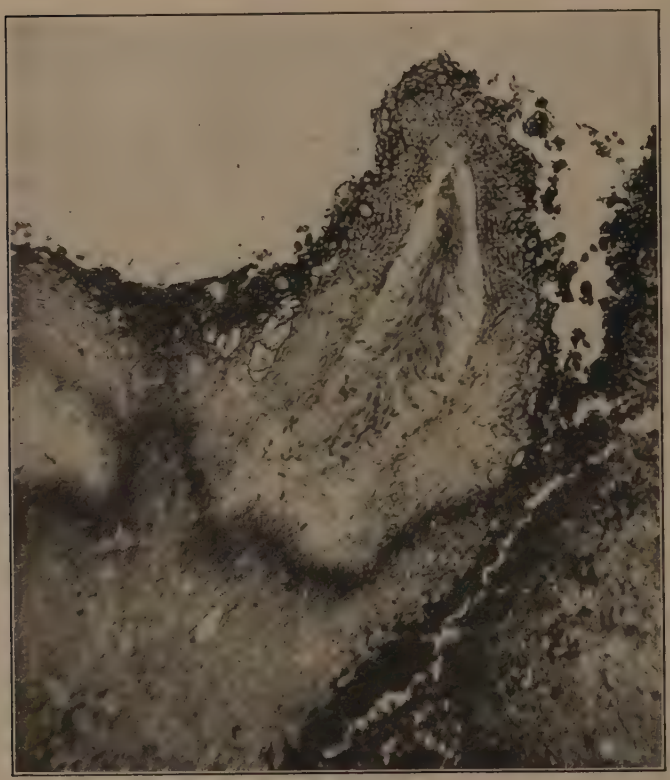
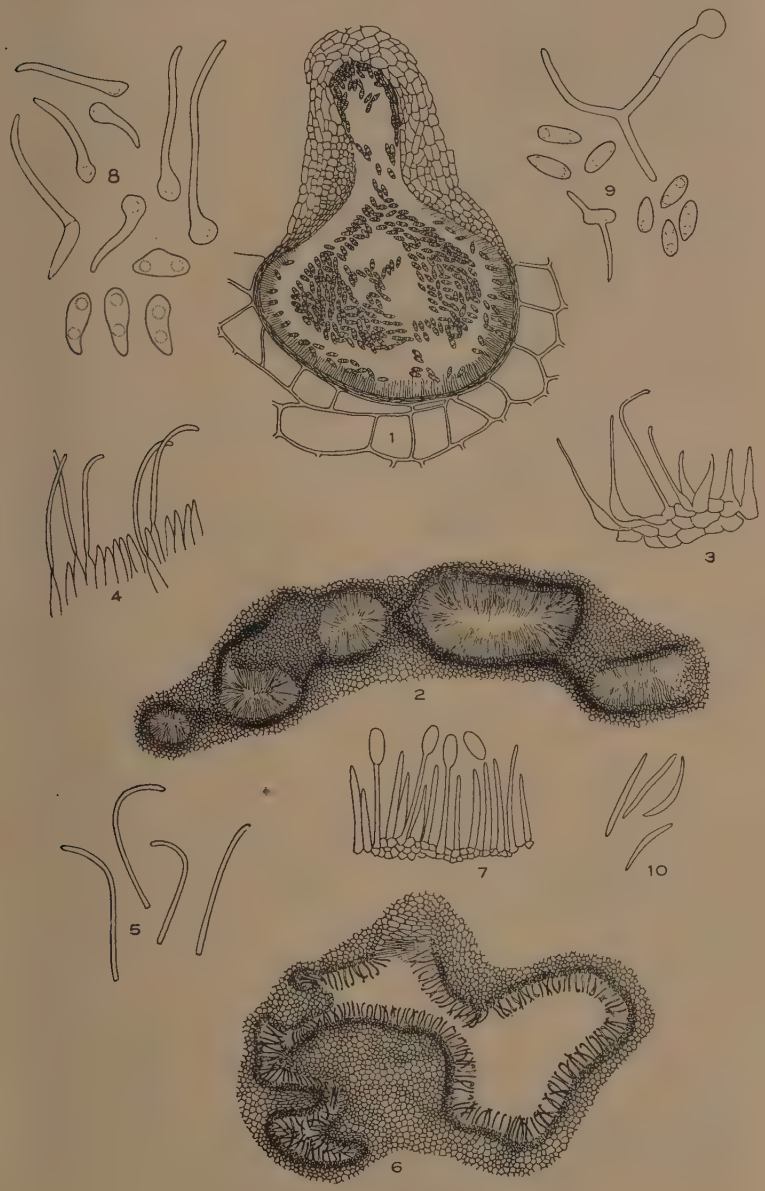
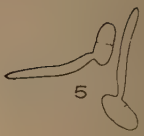
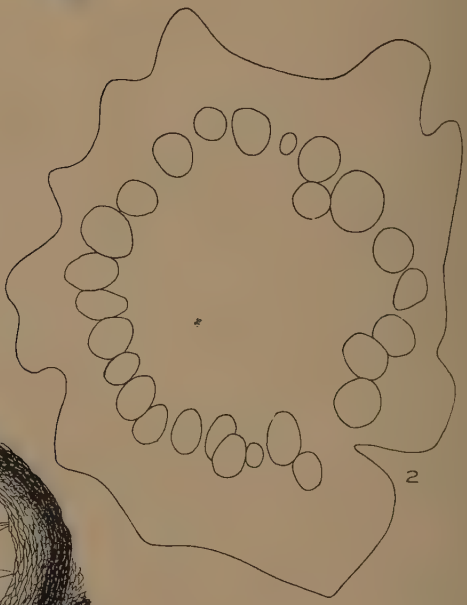


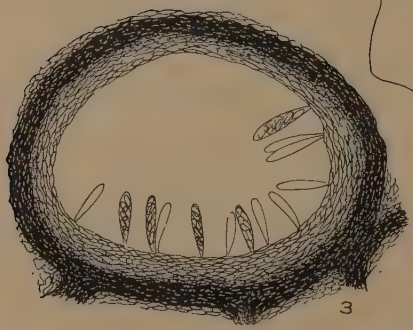
FIG. 2.—A SINGLE PYCNIDIUM FROM FIGURE 1 MORE HIGHLY MAGNIFIED.



PYCNIDIAL STAGE OF DIAPORTHE BATATATIS.







ASCOGENOUS STAGE OF DIAPORTHE BATATATIS.